

present. *Anabaena variabilis* was grown autotrophically, harvested at 1000g for 5min. and disintegrated by 3×45 sec. ultrasonic treatment at 0°, and a crude extract was prepared as previously described (Pearce & Carr, 1967). Incubation of NADPH (0.1 μ mole) in potassium phosphate buffer, pH 7.6 (200 μ moles), in the presence of algal extract (3–6mg. of protein), led to a steady but slow decrease in E_{340} when compared with a non-enzyme or boiled-enzyme control. This rate, which was measured in an Optica CF4R recording spectrophotometer with a scale expanded to 0.2, corresponded to 0.38 ± 0.04 μ mole of NADPH oxidized/min./mg. of protein. Under anaerobic conditions, no oxidation was obtained. When NADPH (0.2 μ mole) was added to a pre-equilibrated extract in a Clark oxygen electrode assembly, an increase in rate of O₂ uptake was recorded; this rate was equivalent to 0.21 μ mole of O₂/min./mg. of protein or 0.42 μ mole of NADPH oxidized/min./mg. of protein.

A smaller rate (0.18 ± 0.03 μ mole/min./mg. of protein) of oxidation was found when NADH was incubated and E_{340} measured. Transhydrogenase activity was not detected under the conditions employed.

Some attention has been directed to proving that these activities were obtained with extracts from pure blue-green algae cultures. For example, before harvesting, samples of grown cultures were inoculated into various yeast-extract solutions as well as on to nutrient agar made up with tap water or with spent autotrophic media. No growth was obtained after incubation in the dark at 30° for 4 weeks. Extracts containing NADPH oxidase, markedly more active than NADH oxidase, have been obtained from *Nostoc muscorum*, *Mastigocladus laminosus*, *Anabaena cylindrica* and *Framyella diplosiphon*. The data present will be discussed in relation to possible mechanisms of autotrophic metabolism.

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The Photometabolism of Benzoic Acid by *Rhodopseudomonas palustris*: A New Pathway of Aromatic Ring Metabolism.

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The aerobic pathways of oxidative breakdown of aromatic compounds by micro-organisms have been well described. The absence of enzymes of these oxidative pathways in *Rhodopseudomonas palustris* grown anaerobically in the light (i.e. photosynthetically) on aromatic substrates has been demonstrated (Hegeman, 1967; Dutton & Evans, 1967). Furthermore, extensive cultural investigations of the organism grown photosynthetically on benzoate failed to detect any of the intermediates of the known oxidative metabolic pathways of benzoate. Coupled with the facts that both light and an absence of molecular oxygen are necessary for the photosynthetic breakdown of benzoate, this indicates that another mode of aromatic breakdown may be employed by *Rhodopseudomonas palustris*.

Isotope-dilution experiments have been conducted on cells grown photosynthetically on benzoate, to identify the intermediates of this pathway. Buffered cell suspensions [3% (wet wt.) in 0.05M-tris-HCl buffer, pH 7.2 (15ml.)] were illuminated under argon with [*carboxy*-¹⁴C]benzoate (22 μ moles, 5 μ C) in the presence of possible intermediates (0.5mg.) for 90min., after which the cells were centrifuged and the acidified supernatant was extracted with ether. This extract was resolved by thin-layer chromatography on silica gel G developed in two dimensions with benzene-dioxan-acetic acid (45:5:2, by vol.). Radioautograms were prepared from the plates. It was found that the cyclohexanol-2-carboxylic acid and pimelic acid spots contained labelled material. Further chromatography on thin-layer plates of the cyclohexanol-2-carboxylic acid spot (silica gel G, six different solvents) and the pimelic acid spot (silica gel G, five solvents; cellulose powder, four solvents) failed to resolve the radioactivity from the respective carrier. After addition of more carrier (200mg.) to the two extracted spots, both were recrystallized five times without loss of specific radioactivity.

Both cyclohexanecarboxylic acid and Δ^1 -cyclohexenecarboxylic acid were also labelled when added to the system. Experiments are in progress to confirm that the radioactivity is associated with these compounds; to date, cyclohexanecarboxylic acid has been run in five solvents on silica gel G plates without separation of the radioactivity. G. D. Hegeman (personal communication) has noted that *Rhodopseudomonas palustris* grown photosynthetically on benzoate are induced to the assimilation of cyclohexanecarboxylate.

We therefore propose the photometabolism of benzoate by *Rhodospseudomonas palustris* occurs by the following pathway:

Benzoate \rightarrow hydrogenated benzoate derivatives \rightarrow cyclohexanol-2-carboxylate \rightarrow [cyclohexanone-2-carboxylate] \rightarrow pimelate.

Further metabolism may be accomplished by β -oxidation.

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The Extracellular Agarase from a *Cytophaga* Species

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A *Cytophaga* species (N.C.M.B. 1327), previously shown to produce intracellular enzymes that cause breakdown of the polysaccharides agarose and porphyran (Turvey & Christison, 1967*a,b*), was used as the source of an extracellular enzyme with similar activity. When the bacterial cells were removed from cultures at the exponential phase of growth, the cell-free supernatant solution had enzyme activity. The enzyme was isolated by precipitation with ammonium sulphate followed by chromatography on DEAE-Sephadex A-50 (OH⁻ form). The latter step was necessary to free the enzyme preparation from associated polysaccharides.

The purified enzyme differed from that obtained by ultrasonic disintegration of the bacterial cells in that it appeared to be a single enzyme. The ratio of its activity on porphyran measured viscometrically to that measured by production of reducing power (Turvey & Christison, 1967*a,b*) was 25:1 compared with the corresponding ratio 1:9 found for the intracellular enzyme preparation. The extracellular enzyme is thus seen to be an endo-enzyme, producing rapid depolymerization of the substrate without production of low-molecular-weight sugars. This type of activity was confirmed by studying the products of its action on porphyran. The major products were oligosaccharides with only small amounts of the disaccharide, neoagarobiose, and no monosaccharides, compared with the action of the intracellular enzyme, which produced some monosaccharides.

The enzyme displays greatest activity to (a) agarose, with decreasing activity to (b) alkali-treated porphyran (Rees, 1961), (c) porphyran, (d) the

alkali-treated galactan sulphate of *Laurencia pinnatifida*, and (e) the native galactan sulphate of *L. pinnatifida* (Bowker & Turvey, 1968*a,b*), in that order. This is consistent with a specificity for substrates containing an alternating sequence of 3-linked D-galactose and 4-linked 3,6-anhydro-L-galactose units, as in agarose. Increasing departure from this type of structure results in lowering of the extent of enzyme action.

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Metabolism of Monofluorobenzoates by Bacterium N.C.I.B. 8250

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Ali, Calley & Hayes (1962) showed that bacterium N.C.I.B. 8250 oxidized *m*- and *p*-fluorobenzoate after growth on benzoate. Calley & Jones (1965) subsequently demonstrated that *o*- and *p*-fluorobenzoate, and possibly *m*-fluorobenzoate, were inducers for the benzoate-oxidizing system in this organism. We have confirmed these observations in the course of investigations into the metabolism of aromatic compounds by bacterium N.C.I.B. 8250 (Kennedy & Fewson, 1968) and have now examined the metabolic fates of the monofluorobenzoates.

None of the monofluorobenzoates was utilized as sole source of carbon and energy for growth of bacterium N.C.I.B. 8250. Cell yield on limiting benzoate, salicylate or succinate, however, was increased by suitable concentrations of *o*-fluorobenzoate. Yield in the presence of *m*-fluorobenzoate was slightly augmented but the appearance of brown material in the medium made estimates of growth difficult. *p*-Fluorobenzoate generally decreased the yield on other carbon sources but was itself metabolized. Both *o*-fluorobenzoate and, to a smaller extent, *m*-fluorobenzoate appeared to be incorporated into cell material since the specific radioactivity of cells grown on [*ring*-U-¹⁴C]-benzoate was decreased in the presence of these two compounds.

Examination of the medium during growth on benzoate + *o*-fluorobenzoate showed the transitory appearance of a compound reacting positively in the

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